SUMMARY

Several genetic manipulation techniques are being actively investigated in a number of fish species. Induced triploidy can be a useful method of generating sterile individuals. Homozygous diploid individuals can be generated and used to rapidly generate homozygous inbred lines. Both induced triploidy and induction of homozygous diploids do not appear to be possible in mammals. Gene transfer technology is just beginning to be developed for fish; it may have a number of valuable applications. Gene banking faces some difficulties in fish that are not present in mammals. Fish egg and embryo cryopreservation has not yet been successful, and new methods may need to be developed to reconstitute fish strains from cryopreserved sperm or embryonic cells.

INTRODUCTION

There has been a tremendous increase in research on genetic manipulation techniques in fish recently. This is due both to the increasing importance of aquaculture in food production (Summerfelt, 1982) and the advantages of fish as experimental animals.

I will review recent developments in the application of four types of "genetic engineering" techniques to fish: induced polyploidy, production of homozygous diploids, gene transfer and gene banking. Possible future research directions and contrasts to corresponding research in mammals will be discussed.

GENETIC ENGINEERING TECHNIQUES

Induced Polyploidy

In contrast to polyploid mammals, triploid and tetraploid fish are frequently viable and normal in external appearance. Triploidy and tetraploidy can be induced using heat shock, cold shock and hydrostatic pressure treatments of fertilized eggs (Purdom, 1983; Thorgaard, 1983). Triploids are produced by treatments soon after fertilization while tetraploids are produced by treatments at the time of the first cleavage division (variable among species).

Induced triploids are of interest in aquaculture for two reasons. Firstly, triploids are sterile and may show improved meat quality and growth at and after the age of sexual maturation (Wolters et al., 1982; Lincoln and Scott, 1984). Secondly, triploid fish hybrids are typically more viable than diploid hybrids (Scheerer and Thorgaard, 1983; Chevassus et al., 1983). This may facilitate combining desirable characters of different fish species.

Production of Homozygous Diploids

Homozygous diploid fish can be produced by first irradiating the sperm and activating a normal egg to develop (gynogenesis), or irradiating the egg and fertilizing with a normal sperm (androgenesis). These treatments are followed
by temperature or pressure treatments to block the first cleavage division. In contrast to the inviability of such individuals in mammals (McGrath and Solter, 1984), homozygous androgenetic and gynogenetic fish can survive, although they have reduced viability due to inbreeding depression (Streisinger et al., 1981; Chourrout, 1984; Parsons and Thorgaard, 1985).

The main application of homozygous diploid fish is likely to be in the generation of homozygous inbred lines. The offspring of a single homozygous male, after androgenesis, or of a single homozygous female, after gynogenesis, will be isogenic. Streisinger et al. (1981) generated homozygous lines of zebra danios using this approach. Such lines are likely to be quite valuable in basic research and might be useful in breeding programs.

Gene Transfer

Gene transfer is a new area of research in fish as well as mammals. Similar approaches are being used in both groups; cloned genes are microinjected into fertilized eggs and hopefully incorporated into the developing embryo. Zhu et al. (1985) recently reported incorporation of a plasmid carrying a human growth hormone gene into the genome of goldfish embryos. A number of other laboratories are also investigating gene transfer in fish by microinjection.

The types of genes to use in such studies are a major concern. Growth hormone genes are an obvious candidate, since supplemental growth hormone has been demonstrated to increase growth rate in some fish (Sekine et al., 1985; Wagner et al., 1985). Other single genes also have the potential to lead to dramatic increases in growth rate, and may be appropriate candidates for transfer. A gene that results in expression of a new phosphoglucomutase locus in the liver of rainbow trout, for example, has been found to increase growth rate (Allendorf et al., 1983). Finally, genes that provide disease resistance often differ between species, and may be good candidates for transfer once their genetic basis is understood and they can be isolated.

An alternate gene transfer approach that has been attempted in fish involves the production of gynogenetic individuals with two chromosome sets from the female parent plus chromosome fragments from the irradiated sperm of the male parent (Thorgaard et al., 1985). This approach, if successful, might provide an alternative transfer approach for genes which had not been cloned if they could be selected for in the offspring. Results to date indicate that genes on the paternal chromosome fragments may be expressed but that the chromosome fragments are lost at a high rate during mitotic cell division.

Gene Banking

The need from a conservation standpoint to preserve a variety of strains of the different fish species is obvious. These strains could also serve as a genetic reservoir for the future improvement of domestic strains. Many distinct strains of economically important fish species are known to exist and to have important genetic differences (Berst and Simon, 1981).

Current gene banking technology is unfortunately inadequate for full preservation of fish strains by cryopreservation. In contrast to the mammalian situation in which sperm, eggs and embryos can all be cryopreserved (Leibo, 1981) only sperm cryopreservation has been successful to date in fish (Stoss, 1983). This is probably related to the large size of fish eggs and embryos and associated difficulties with cryoprotectant penetration and cooling rates (Stoss, 1983).
An important task for the future will be to develop approaches that will allow full conservation of fish strains by cryopreservation, since the current technology would require repeated backcrossing to another strain to reconstitute from cryopreserved sperm. Possible approaches could include nuclear transplantation with frozen embryonic cells and androgenesis in which two separate cryopreserved sperm were incorporated into an embryo. Given the successful results with fish nuclear transplantation (Gasaryan et al., 1979) and androgenesis (Parsons and Thorgaard, 1985) it appears likely that these approaches could succeed.

CONCLUSIONS

Although much less effort has been applied to genetic manipulation in fish than in mammals, considerable progress has been made in several areas. Induced polyploidy and the generation of homozygous diploids have already been shown to be feasible and to have application in the generation of sterile individuals and inbred lines, respectively. Gene transfer technology is just now being developed, and is likely to have numerous applications. Improved gene banking technologies will be important in order to allow cryopreservation and reconstitution of genetically distinct strains of fish.

REFERENCES


